On the autonomic nervous and chromaffin control systems of lungfish

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ABSTRACT

There are currently three extant genera of true lungfish (dipnoans): one in South America (*Lepidosiren*), one in Africa (*Protopterus*) and one in Australia (*Neoceratodus*). Although there are numerous fish that can breathe air, a common feature of the dipnoans is a vascular arrangement with a pulmonary artery, and a pulmonary vein that brings the oxygenated blood directly to the heart.

The control systems regulating the blood flow in the dipnoan circulation are not well known. In most vertebrates, adrenergic systems – circulating catecholamines and adrenergic nerves (postganglionic neurons of the sympathetic nervous system) – play essential roles in the control of both cardiac function and vascular resistance. In lungfish there is little evidence for adrenergic nerves, instead there is an impressive presence of chromaffin cells within the atrium of the heart, in the walls of the left posterior cardinal vein (azygos vein) and the intercostals arteries. A cholinergic innervation of the lung, the heart and possible some vascular segments has been demonstrated in *Lepidosiren* and *Protopterus*, but any antagonistic adrenergic control is likely to be due to locally released ("paracrine") or circulating catecholamines from the chromaffin stores in the heart and vascular walls.

Key words: Lungfish, dipnoans, autonomic innervations, chromaffin system

Introduction

The term "autonomic nervous system" was coined by Langley (1898) for the system of cranial and spinal motor nerves in the vertebrate body that control visceral organs (in a wide sense), and also the intrinsic neurons of the gut. He divided the autonomic nervous system into three divisions: the sympathetic, the parasympathetic and the enteric system. In mammals, the sympathetic pathways leave the spinal cord in the thoracic and lumbar segments, while the parasympathetic pathways either run in the cranial nerves, or in the sacral segments. The enteric nervous system is made up from the intrinsic neurons of the gut, and it is generally difficult to distinguish the autonomic fibres (which, by definition are motor nerves) from a variety of sensory pathways (which are thus not formally part of the autonomic nervous system). In the non-mammalian vertebrates, there is also a problem with separating the sympathetic from the parasympathetic pathways that emerge from the spinal cord (see also Nilsson 1983 and Gibbins 1994).

The term "chromaffin", on the other hand, was used by Kohn (1902) for cells that turned brown or black in response to dichromate solution. The reaction is due to formation of adrenochromes due to oxidation of catecholamines in the cells (see Santer 1994). Modern techniques, notably the formaldehyde-induced formation of fluorescent compounds from cellular catecholamines are much more sensitive (the Falck-Hillarp technique: Eränkö 1955; Falck and Owman 1965). Although the levels of adrenaline and noradrenaline may be too low to be detected with chromate solution, non-neuronal cells that show catecholamine storage are usually designated "chromaffin".

So, how about these systems in the lungfish? The three extant genera of lungfish are *Lepidosiren (paradoxa)* in South America, *Protopterus* in Africa (of which there are several species: *P. aethiopicus, P. amphibius, P. annectens, and P. dolloi*) and *Neoceratodus (forsteri)* in Australia. The American and African lungfish look fairly similar, and also share the quite embarrassing (for a fish) characteristic of being obligate air breathers – they will drown if prevented from breathing air. On the other hand, both these lungfish can aestivate, buried in mud at near-dry condition for extended periods, relying on air breathing. The Australian lungfish can survive on either water- or air-breathing, and although it is thus perhaps more "fish-like" that the others, it shows many anatomical similarities with early Devonian lungfish fossils (*e.g.*, Romer 1962).

It must be pointed out, that while taxonomists appear to agree on the close relationship between the three genera, especially between *Protopterus* and *Lepidosiren*, any generalised view of the group "lungfish" such as expressed in this compilation, should be regarded with at least one pinch of salt.

While there are numerous splendid studies of the cardiovascular, pulmonary and other events in lungfish, the systems responsible for the physiological control have received comparatively little attention. This brief review is an attempt to summarise some points of interest in the control of the heart, vasculature and lung of dipnoans, based on a description of the functional anatomy of the autonomic nervous and chromaffin systems. More comprehensive reviews are those by Nilsson and Holmgren (1992) and Axelsson (1993). Sadly, the development of the field since these reviews has not been overwhelming.

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Anatomical considerations

The autonomic nervous system in lungfish

The sympathetic chains in lungfish are not impressive, and were overlooked in 19th century studies. Giacomini (1906) who first described the sympathetic chains in *Protopterus*, must have been really pleased to find them, happily quoting *verbatim* the texts by Wiedersheim (in German) and Parker (in English) who couldn't find any (see Giacomini 1906).

A good desciption was made by Jenkin (1928) of the sympathetic chains in *Lepidosiren*. The parasympathetic portion of the autonomic nervous system is better developed. In *Protopterus* and *Lepidosiren* there are substantial branches of the vagus nerve (X) to the heart, lung and gut, and this is also true for *Neoceratodus* in which there may also be an autonomic component in the oculomotor nerve (III) (Nicol 1952).

Immunohistochemical studies show the presence of several neuropeptides in neurons within the gut wall of *Lepidosiren*, *Protopterus* and *Neoceratodus*), demonstrating an enteric nervous system. These neuropeptides include enkephalin, galanin, gastrin/cholecystokinin, neurotensin, neuropeptide Y, somatostatin, substance P and vasoactive intestinal polypeptide (VIP). In addition, neurons showing immunoreactivity to the amine 5-hydroxytryptamine (serotonin) were demonstrated (Nilsson and Holmgren 1992; Holmgren at al. 1994).

A summary of the general anatomy of the autonomic nervous system of lungfish, based primarily on the descriptions made by Jenkin (1928), is presented in Fig. 1.

The chromaffin system in lungfish

Chromaffin cells were described by Giacomini (1906) in the left cardinal vein ("azygos vein") of *Protopterus*, and also in the walls of the segmental intercostal arteries. The latter observation was confirmed by Holmes (1950), and our own studies of *Protopterus* using the Falck-Hillarp technique demonstrated masses of catecholamine-storing cells, capable of catecholamine synthesis in the wall of the azygos vein, the intercostal arteries, and also lining the atrium of the heart (Abrahamsson et al. 1979a; Abrahamsson et al. 1979c). An innervation of the intracardiac cells was postulated (Scheuermann 1979), as was storage of dopamine in these cells (Scheuermann et al. 1981).

The Falck-Hillarp technique, and the somewhat related glyoxylic acid technique (Furness and Costa 1975), were used to demonstrate intra-atrial chromaffin tissue also in *Lepidosiren* (Axelsson et al. 1989) and *Neoceratodus* (Fritsche et al. 1993). See also Fig 2. A study of the

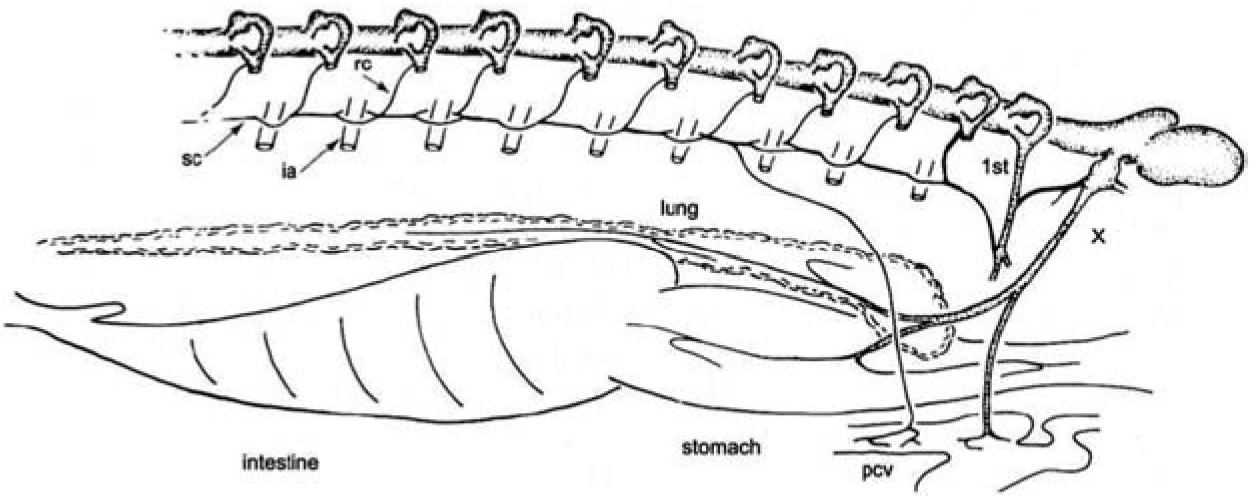

Figure 1. A simplified view of the arrangement of the autonomic nervous system of lungfish, based primarily on the descriptionsof *Lepidosiren paradoxa* by Jenkin, 1928. Legend: 1st; first pair of spinal nerves; ia, intercostal artery; pcv, posterior cardinal vein ("azygos vein"); rc, ramus communicans; sc, sympathetic chain; X, vagus nerve. Reproduced with permission from Nilsson and Holmgren, 1992 (Marcel Dekker Inc).

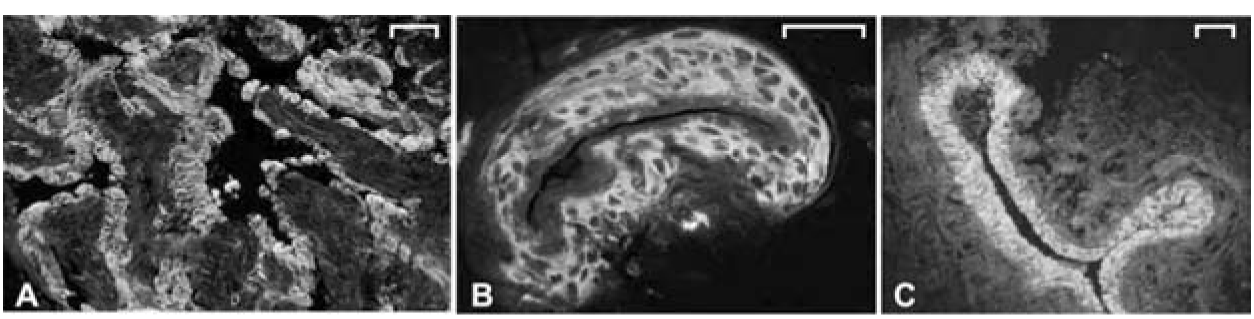

Figure 2. Formaldehyde-induced fluorescence showing cateholamine stores in chromaffin cells from *Protopterus aethiopicus*. A. Cathecholamine-storing cells intermingled with cardiac muscle of the atrium. B. Dough-nut shaped chromaffin tissue in the wall of an intercostal artery. C. Chromaffin tissue lining the wall of the posterior cardinal vein (azygos vein"). Clibration bars in all figures = 100 µm.

ultrastructure of *Neoceratodus* heart pinpoints the positions of the catecholamine-storing intracardiac cells (Chopin and Bennett 1995).

Control mechanisms

The heart

A cholinergic, inhibitory innervation of the lungfish heart has been concluded from studies in all three lungfish genera, although only in *Lepidosiren* has a cholinergic tonus on the heart been demonstrated. In *Lepidosiren*, the heart rate increases slightly in response to atropine, and the fright response (transient cardiac arrest or bradycardia) disappears after atropine in both *Lepidosiren* and *Neoceratodus* (Axelsson et al. 1989; Fritsche et al. 1993).

An adrenergic control of the heart, most likely due to the intracardiac stores of catecholaimes in chromaffin cells, has been shown in *Lepidosiren*, where there is also an adrenergic tonus responsible for an increase in basic heart rate (Axelsson et al. 1989). However, injection of adrenaline *in vivo* has little or no effect on the heart in any lungfish studied, nor is the nature of control of the intracardiac chromaffin cells well understood.

The lung

The anatomy of the dipnoan lung has long attracted interest – the animals are, after all, called lungfish. Detailed accounts include that of Grigg (1965) working with *Neoceratodus*. Apart from the obvious interest in the control of blood flow, the direct nervous control of the lung itself has received some attention. In *Protopterus*, electrical stimulation of the pulmonary branches of the vagus nerve elicits contractions of the lung wall, and these contractions can be modified by application of drugs that are known to affect cholinergic nerve function. Thus, the cholinesterase inhibitor eserine enhances the contractions, while both d-tubocurarine and atropine abolish the response (Fig 3). The effect of the "ganglionic blocker" d-tubocurarine suggests a ganglionic synapse in the vagal innervation of the lung wall (Abrahamsson et al. 1979b)

The vasculature

The most interesting region when discussing control mechanisms of the lungfish vasculature is, perhaps, the vessels affecting the blood flow into the lung: the *ductus arteriosus* and the pulmonary arterial vascular segment (PAVS). The two most anterior pairs of efferent branchial

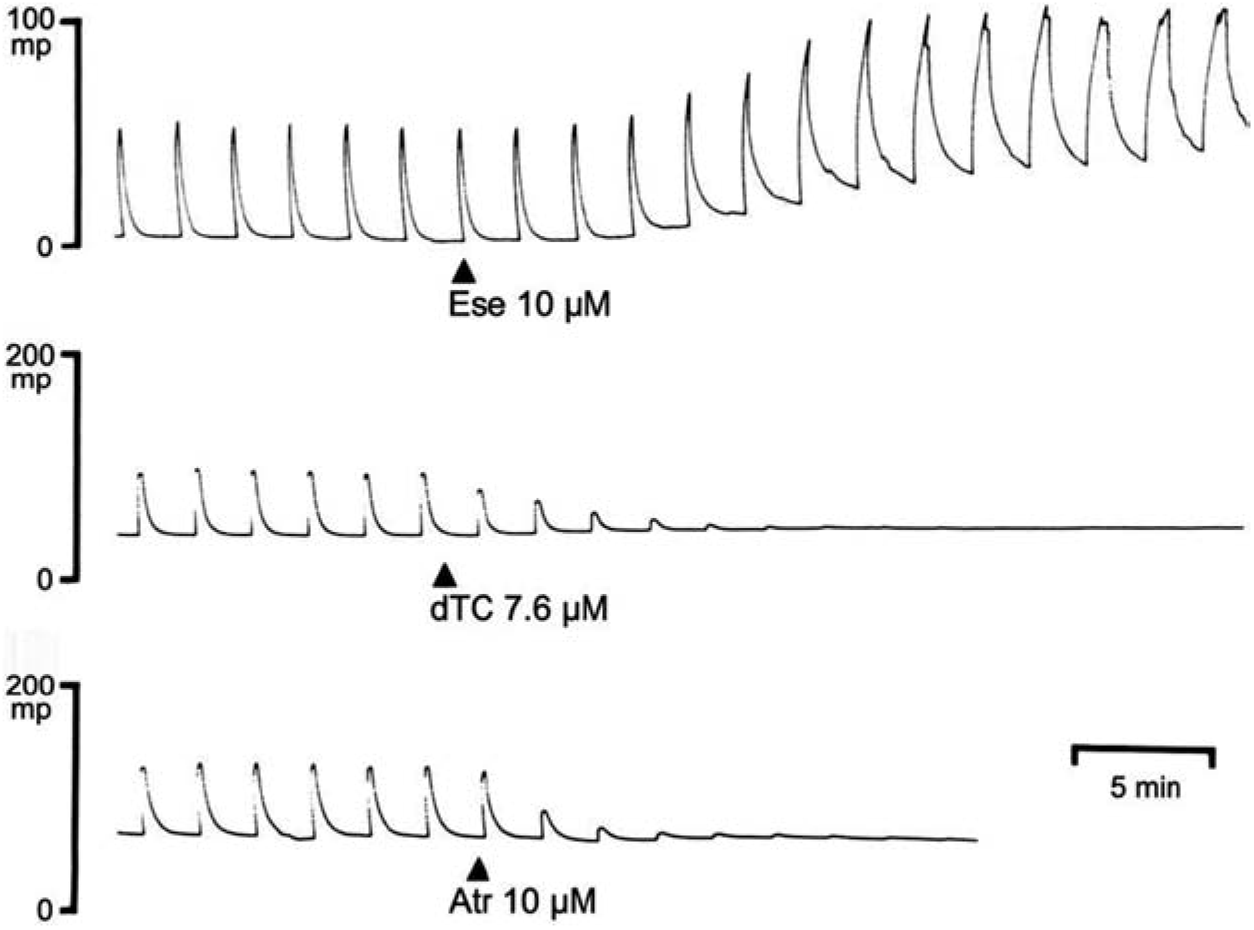

Figure 3. Contractions of the lung wall caused by electrical stimulation of the pulmonary branch of the vagus nerve in *Protopterus aethiopicus.* Addition of the cholinesterase inhibitor eserine (physostigmine) (Ese) enhances the contractions, while both the nicotinic cholinoceptor antagonist d-tubocurarine (dTC) and the muscarinic cholinoceptor antagonist atropine (Atr) abolish the response. Reproduced with permission from Abrahamsson et al., 1979b (Wiley-Blackwell Publishing Ltd)

arteries join to form the dorsal aorta, and the respiratory function of the corresponding gill arches is poor. The respiratory function of the two posterior pairs of gill arches is much better, and their efferent branchial arteries join to form the pulmonary artery. Gill shunts that would allow blood to bypass the respiratory parts in the posterior gill arches was suggested by Laurent et al. (1978), but their control remains unknown.

The *ductus* is a short connection between the dorsal aorta and the pulmonary artery (see also Fig 4). It is dilated by acetylcholine, and may be controlled by cholinergic fibres – possibly of vagal origin. Noradrenaline or hypoxia, on the other hand, constrict the *ductus* (Fishman et al. 1985; see also discussion by Axelsson 1993). The PAVS regulates blood flow into the lung, is constricted by acetylcholine and may also be controlled by cholinergic constrictor fibres – again possibly of vagal origin (Fishman et al. 1985).

A comprehensive hypothesis for the redirection of blood flow in air-breathing compared to water-breathing lungfish was developed by Fishman et al. (1985). In this model, blood returning from the body to the heart during water-breathing enters the functional posterior gill arches, and blood oxygenated from the water continues via an open *ductus* to the dorsal aorta and on to the tissues. The PAVS is kept closed.

During periods of aquatic hypoxia, and thus air-breathing, the gill shunt (Laurent et al. 1978) opens, allowing blood ejected by the heart to bypass the respiratory surfaces of the now non-functional posterior gills. Blood is then diverted by the closed *ductus* via the open PAVS into the lung, returning to the heart via the pulmonary vein for a "second round" via the anterior gill arches into the dorsal aorta.

Adding the fragmentary information available about the putative control of the effectors, it may perhaps be concluded that while there is no clear information about the control of the gill shunt, a model for the regulation of blood flow via the *ductus* and PAVS may be constructed. Thus, it appears that a cholinergic, possibly vagal, control of the two sphincters would favour the blood flow pattern during water-breathing (dilation of the *ductus* and constriction of the PAVS) (Fig. 4).

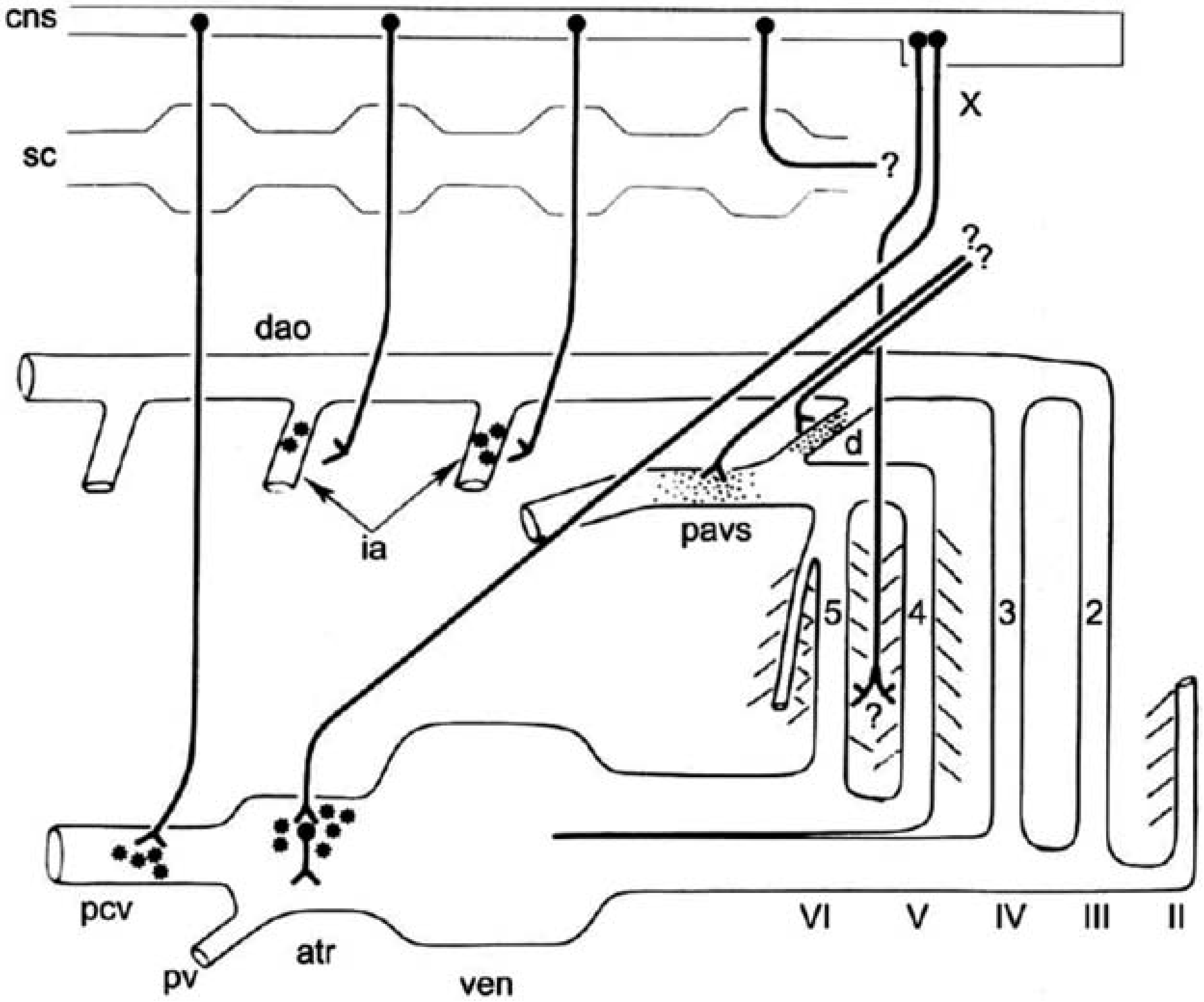

Figure 4. Hypothetical "working model" of cardiovascular control functions in lungfish. Legend: atr, atrium; csn, central nervous system; d, ductus arteriosus; dao, dorsal aorta; ia, intercostal artery; pavs, pulmonary arterial vascular segment; pcv, posterior cardinal vein ("azygos vein"); pv, pulmonary vein; rc, ramus communicans; sc, sympathetic chain; ven, ventricle; X, vagus nerve. Arabic numbers 2-5 refer to the gill arteries, while the Roman numbers II-VI refer to the branchial arches. Reproduced with permission from Nilsson and Holmgren, 1992 (Marcel Dekker Inc).

Acknowledgments

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Figure 5. Gordon C. Grigg, probably not looking for the Queensland lungfish, *Neoceratodus forsteri.*

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